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THIAMINE TRANSPORT IN THE BRUSH BORDER MEMBRANE VESICLES OF THE GUINEA-PIG JEJUNUM

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Summary

Transport of [^{35}S]thiamine was studied with membrane vesicles prepared from the brush border of guinea-pig jejunum, in which thiamine pyrophosphokinase (EC 2.7.6.2) was not detected.

The presence of an Na^+ gradient from outside to inside of the vesicles did not affect thiamine transport, whereas L-proline uptake into the vesicles of the same preparation was stimulated under identical conditions.

The equilibrium level of thiamine uptake decreased with increasing osmolarity of the medium, which indicates that thiamine is transported into the membrane vesicles.

The initial rate (30 s) of thiamine uptake increased linearly with increasing thiamine concentration throughout the range from 0.06 to 10 μM in the medium, in the presence and absence of an Na^+ gradient. No effect of other monovalent cations, including K^+, Li^+ and choline$^+$, was observed on thiamine transport.

Pyrithiamine, an antimetabolite of thiamine, and unlabeled thiamine, both added in very excessive amounts, did not inhibit labeled thiamine transport into the membrane vesicles.

These results confirm the assumption that thiamine passes through the brush border membrane of guinea-pig jejunum by simple diffusion.

Introduction

Intestinal thiamine transport has been studied by various investigators using different animals [1,2]. The results of some studies suggest that absorption

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

of thiamine is an active process at low concentrations (less than approx. 2 μM), while diffusion is involved in absorption at high concentrations (greater than approx. 2 μM) [3—5]. However, these studies were made using the everted sac method or the tissue accumulation method. When thiamine transport is measured by these methods, coupling of thiamine metabolism to the transport process, especially conversion of thiamine to thiamine pyrophosphate in the cytoplasm catalyzed by thiamine pyrophosphokinase, should be taken into consideration. It is, therefore, very difficult to examine thiamine transport through the brush border membrane itself of the small intestinal epithelium by these methods.

Membrane vesicles from the intestinal brush border membrane have been recently used to study Na^+-dependent transport of amino acids [6,7] and L-ascorbate [8] as well as D-glucose [9—11].

To avoid coupling of the metabolism to thiamine transport and also to investigate the mechanism of thiamine entry through the membrane, brush border membrane vesicles prepared from guinea-pig jejunum were used in this study, in which no thiamine pyrophosphokinase was present and an Na^+ gradient-dependent L-proline uptake system was confirmed to be present. This paper presents results which suggest that thiamine is transported by simple diffusion and that a specific carrier system is not involved in thiamine transport in the small intestinal brush border membrane of the guinea-pig.

Materials and Methods

Isolation of brush border membrane vesicles. Membrane vesicles were prepared from guinea-pig jejunum according to the procedure of Fujita et al. [12]; this procedure has been described previously [7]. This membrane vesicle preparation showed an alkaline phosphatase enrichment and a sucrase enrichment about 10-times greater than that of the starting homogenates.

Membrane vesicles were finally suspended in a medium composed of 100 mM D-mannitol, 0.1 mM $MgSO_4$, and 5 mM Tris-Hepes (pH 7.5).

Assay of transport activity. All assays of transport activity were carried out at 25°C according to the procedure used for measuring L-proline transport activity in membrane vesicles of guinea-pig ileum as described previously [7]. Membrane vesicles were incubated in a medium containing 100 mM D-mannitol, 0.1 mM $MgSO_4$, 5 mM Tris-Hepes (pH 7.5) and labeled substrate, either [^{35}S]thiamine or L-[^{14}C]proline. Other additions are described in the table and figure legends. The transport of substrate was terminated by diluting an aliquot of the sample (approx. 100 μg of membrane protein) with a 40-fold vol. of an ice-cold buffer composed of 150 mM NaCl, 50 mM $MgCl_2$, 30 mM D-mannitol, and 10 mM Tris-Hepes (pH 7.5). The diluted aliquot was immediately filtered through a Millipore filter (HA 025, 0.45 μm) and washed once with 3 ml of the same ice-cold buffer. Radioactivity retained on the filter was counted in Bray's liquid scintillation fluid.

All assays were performed in triplicate with freshly prepared membrane vesicles and each assay was repeated at least three times with different membrane preparations. Protein was determined according to the method of Lowry et al. [13].

Chromatographic analysis of [^{35}S]thiamine taken up by membrane vesicles. [^{35}S]Thiamine transported into the membrane vesicles was analyzed by paper chromatography according to a procedure described previously [14].

Determination of thiamine pyrophosphokinase activity in brush border membrane vesicles. The reaction medium contained in 1 ml : 2 mM ATP, 5 mM $MgSO_4$, 10 μM thiamine, 10 mM Tris-Hepes (pH 7.4), and membrane vesicles (about 1 mg of protein). After incubation for 30 min at 37°C, the reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid and then centrifuged. The supernatant obtained was subjected to high-performance liquid chromatography after conversion of thiamine and thiamine pyrophosphate, if formed, to thiochrome and thiochrome pyrophosphate as described [15].

Chemicals. All reagents were of the highest purity commercially available. L-[U-^{14}C]Proline (295 mCi/mmol) and [^{35}S]thiamine hydrochloride (1619 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, U.K. and pyrithiamine hydrobromide from Sigma.

Results

Time course of Na^+-gradient-dependent transport of L-proline. To confirm the presence of the L-proline transport system in the membrane vesicles as shown in a previous paper [7], the time course of L-proline transport into the membrane vesicles was examined (Fig. 1). In the absence of an Na^+ gradient, a steady-state level of transport was reached in about 30 min. The presence of

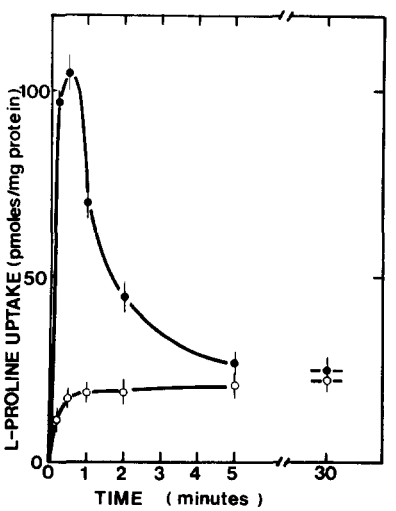

Fig. 1. Effect of Na^+ on L-proline transport in brush border membrane vesicles of guinea-pig jejunum. Membrane vesicles were suspended in a medium containing 100 mM D-mannitol, 0.1 mM $MgSO_4$, and 5 mM Tris-Hepes (pH 7.5). L-Proline transport was initiated by adding 50 μl of the membrane suspension to 50 μl of an incubation medium composed of 40 μM L-[U-^{14}C]proline, 100 mM D-mannitol, 0.1 mM $MgSO_4$, 5 mM Tris-Hepes (pH 7.5) and 200 mM NaCl. Both the membrane suspension and the incubation medium were preincubated independently at 25°C before mixing, followed by further incubation at 25°C. As control, NaCl in the incubation medium was replaced by 200 mM KCl. NaCl (●——●); KCl (○——○). Each point represents mean ± S.D.

an Na^+ gradient from outside to inside of the membrane vesicles stimulated L-proline transport and showed a typical 'overshoot' phenomenon of transport.

These results clearly indicate the presence of an active transport system for L-proline in brush border membrane vesicles prepared from guinea-pig jejunum and suggest that the membrane vesicles are useful in transport studies of thiamine.

Thiamine pyrophosphokinase activity in brush border membrane vesicles. When thiamine pyrophosphokinase activity was measured using the procedure described in Materials and Methods, the reaction product, thiamine pyrophosphate, was not detected (data not shown). This analytical procedure is sufficient to detect 0.05 pmol of either thiamine or thiamine pyrophosphate. Therefore, if approx. 0.1% of thiamine used as substrate in the reaction mixture were to be converted to thiamine pyrophosphate, it would be easily detected at this sensitivity without concentrating the reaction product. Nevertheless, that thiamine pyrophosphate was not detected under the conditions employed suggests that thiamine pyrophosphokinase is not present in membrane vesicles.

Time course of thiamine transport. The transport of thiamine into the membrane vesicles as a function of incubation time is shown in Fig. 2. There was no significant difference between the transport in the presence of an Na^+ gradient (out > in) and in its absence and also a typical overshoot phenomenon observed in L-proline uptake (Fig. 1) was not seen.

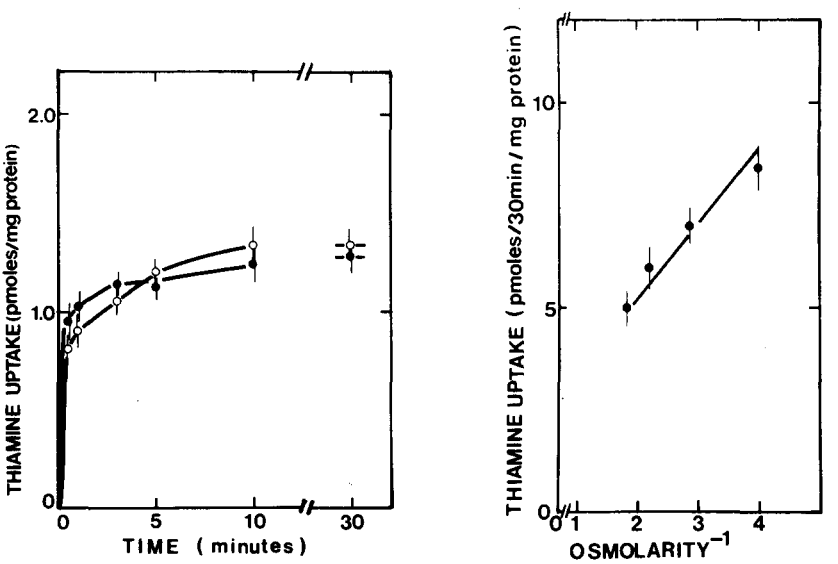

Fig. 2. Time course of thiamine transport. The time course of thiamine transport was measured under the same conditions as described in the legend of Fig. 1, except for the use of 0.25 μM [^{35}S]thiamine (final concentration) as transport substrate. NaCl (●——●); KCl (○——○). Each point represents mean ± S.D.

Fig. 3. Effect of medium osmolarity on thiamine transport. Thiamine transport was measured 30 min after incubation in a medium containing 1.0 μM [^{35}S]thiamine, 0.1 mM $MgSO_4$, 5 mM Tris-Hepes (pH 7.5) and 25 mM KCl (final concentrations) and D-mannitol was added to the medium to give the indicated osmolarities. Each point represents mean ± S.D.

These results suggest that thiamine transport into membrane vesicles is not dependent on an Na^+ gradient.

Effect of medium osmolarity on the transport of thiamine. The relationship between medium osmolarity and thiamine transport was studied (Fig. 3). When medium osmolarity was increased by increasing D-mannitol concentrations, the uptake of thiamine decreased inversely. This indicates that thiamine was transported into the intravesicular space.

Thiamine taken up by the vesicles was found to exist as the non-phosphorylated form when analyzed by paper chromatographic methods [14] as expected by the lack of thiamine pyrophosphokinase in the vesicles.

Effect of thiamine concentration on the initial rate of transport. The effect of different concentrations of thiamine on the initial rate of uptake is illustrated in Figs. 4 and 5. The rate of thiamine transport increased linearly with increasing thiamine concentrations throughout the range from 0.06 to 10 μM both in the presence and absence of an Na^+ gradient.

These results suggest that thiamine is transported into the membrane visicles by simple diffusion even at thiamine concentrations lower than 1 μM.

Effect of monovalent cations on thiamine transport. Table I shows the effect of monovalent cations on the initial rate of thiamine transport. The rate was not affected by these cations at high (2.0 μM) and low (0.25 μM) thiamine concentrations nor by a decrease in the concentration of NaCl in the medium.

Effect of a thiamine analogue and unlabeled thiamine on [^{35}S]thiamine transport. To confirm the assumption of thiamine transport by a simple diffusion mechanism (Figs. 4 and 5), the effect of pyrithiamine, an antimetabolite

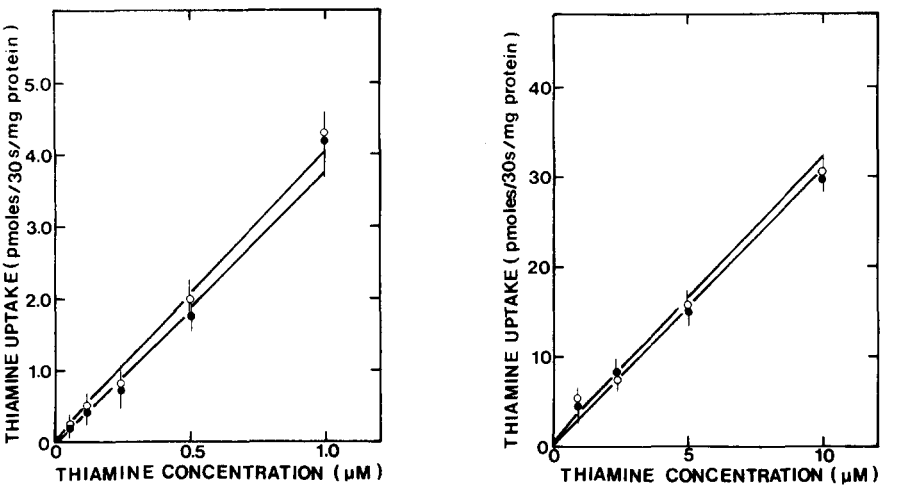

Fig. 4. Effect of low thiamine concentrations on the initial rate of transport. The transport for 30 s was measured in a medium containing [^{35}S]thiamine at the indicated concentration, 100 mM D-mannitol, 0.1 mM $MgSO_4$ and 5 mM Tris-Hepes (pH 7.5) supplemented with either 100 mM NaCl (●———●) or 100 mM KCl (○———○) (final concentrations). Each point represents mean ± S.D.

Fig. 5. Effect of high thiamine concentrations on the initial rate of transport. The transport for 30 s was measured under the same conditions as described in the legend of Fig. 4, except for the use of high thiamine concentrations. NaCl (●———●); KCl (○———○). Each point represents mean ± S.D.

TABLE I

EFFECT OF MONOVALENT CATIONS ON THIAMINE TRANSPORT

The transport for 30 s was measured in a medium containing 0.25 or 2.0 μM [^{35}S]thiamine, 100 mM D-mannitol, 0.1 mM $MgSO_4$, and 5 mM Tris-Hepes (pH 7.5) supplemented with 100 mM NaCl, 100 mM KCl, 100 mM LiCl, or 100 mM choline chloride (final concentrations). When the NaCl concentration in the medium was changed, the osmolarity of the medium was adjusted to be constant by adding D-mannitol. All values obtained for the uptake are given as mean ±S.D. Results are expressed as pmol [^{35}S]-thiamine incorporated/30 s per mg protein

Cations	Concentration (mM)	[^{35}S]Thiamine concentration	
		Low (0.25 μM)	High (2.0 μM)
Na^+	100	1.11 ± 0.14	8.80 ± 0.86
Na^+	50	0.94 ± 0.88	9.20 ± 0.66
Na^+	25	1.25 ± 0.25	9.76 ± 1.10
K^+	100	1.18 ± 0.20	8.62 ± 1.24
Li^+	100	1.24 ± 0.50	9.50 ± 0.40
$Choline^+$	100	1.01 ± 0.10	8.76 ± 0.70

of thiamine, and of non-labeled thiamine on [^{35}S]thiamine transport was examined (Table II). The presence of a 40- and a 400-fold excess of pyrithiamine produced no decrease in the uptake of 0.25 μM [^{35}S]thiamine. Identical results were obtained by the addition of a 40- and a 400-fold excess of unlabeled thiamine.

These results indicate that a specific carrier system is not involved in thiamine transport in brush border membrane vesicles of guinea-pig jejunum and support the notion that thiamine is transported into the membrane vesicles by simple diffusion.

Thiamine transport in brush border membrane vesicles prepared from the ileum and that at a different temperature. The results presented above were obtained on brush border membrane vesicles of guinea-pig jejunum at 25°C. Identical results were obtained with brush border membrane vesicles of guinea-pig ileum, and experiments carried out at 37°C with membrane vesicles either

TABLE II

EFFECT OF A THIAMINE ANALOGUE AND UNLABELED THIAMINE ON [^{35}S]THIAMINE TRANSPORT

The transport for 30 s was measured in a medium containing 0.25 μM [^{35}S]thiamine, 100 mM D-mannitol, 0.1 mM $MgSO_4$, and 5 mM Tris-Hepes (pH 7.5) supplemented with 100 mM NaCl (final concentrations). Pyrithiamine and unlabeled thiamine added were 10 and 100 μM (final concentrations). All values obtained for the uptake are given as mean ± S.D.

Additions	[^{35}S]Thiamine uptake (pmol/30 s per mg protein)
None	1.25 ± 0.32
Pyrithiamine (10 μM)	1.20 ± 0.05
Pyrithiamine (100 μM)	1.05 ± 0.43
Thiamine (10 μM)	1.09 ± 0.14
Thiamine (100 μM)	1.39 ± 0.22

from the jejunum or ileum produced the same results as those obtained at 25°C (data not shown).

Discussion

The data presented in this paper indicate that thiamine is transported by simple diffusion and, therefore, no specific carrier system participates in thiamine transport in the brush border membrane of guinea-pig jejunum.

According to previous investigations, it has been generally accepted that thiamine transport in the small intestine in animals is mediated by two mechanisms. At high concentrations (greater than approx. 2 μM), thiamine is transported by simple diffusion, whereas at low concentrations (less than approx. 2 μM) it is absorbed by active transport [3—5]. However, such a conclusion is not consistent with our present data obtained with brush border membrane vesicles of guinea-pig jejunum, which indicate a simple diffusion mechanism of thiamine transport even at substrate concentrations as low as 0.06—1.0 μM (Fig. 4).

Although Ferrari et al. [16] have suggested an Na^+ dependency of thiamine transport with everted sacs of rat intestine, our preparation of membrane vesicles, in which the presence of an Na^+-dependent L-proline transport system was clearly demonstrated (Fig. 1), did not manifest such a dependency of thiamine transport on Na^+ (Fig. 2) nor on other cations such as K^+ and Li^+ (Table I). To demonstrate the involvement of a thiamine carrier in the transport, thiamine analogues such as pyrithiamine and chloroethylthiamine were found to inhibit the transport of thiamine [3,4]. However, a 400-fold excess of pyrithiamine did not inhibit thiamine transport into the membrane vesicles and identical results were obtained when a 400-fold excess of unlabeled thiamine was added (Table II). The data obtained with thiamine analogues also did not indicate the involvement of a carrier system for thiamine uptake.

The brush border membrane vesicles used in the present experiments contain amino acid carrier systems, including L-alanine, α-(methylamino)isobutyrate, L-cysteine and L-leucine (data not shown), in addition to L-proline, which were driven either totally or partially by an Na^+ electrochemical potential. It is therefore reasonable to assume that thiamine carrier or thiamine pyrophosphokinase was not lost from the vesicles during the preparation procedure. This is supported by the fact that in our preparation marker enzymes existing in the brush border membrane such as sucrase and alkaline phosphatase were purified approx. 10-fold.

The relation of thiamine transport across the brush border membrane and its pyrophosphorylation to thiamine pyrophosphate has not yet been clarified [1]. Several determined parameters support the idea that a close relationship between transport and phosphorylation appears likely [17—19], whereas a role of phosphorylation in transport has been questioned in other studies [4,16,20]. There were technical difficulties in resolving this relation with everted intestinal sacs or rings.

To avoid technical problems in the study of the intestinal transport of thiamine and to examine the transport across the brush border membrane, vesicles which do not contain thiamine pyrophosphokinase were used in this

study and thiamine transport for 30 s was mostly determined. The results obtained indicate clearly that thiamine is transported through the brush border membrane of guinea-pig small intestine by simple diffusion without involvement of a specific carrier system. The results also ruled out the possibility that a group translocation mechanism of thiamine transport is involved as suggested for purine transport in animal cells [21], since thiamine pyrophosphokinase, which is a possible enzyme catalyzing thiamine group translocation, is not present in the vesicles.

After passing through the brush border membrane by simple diffusion, thiamine may be accumulated as thiamine pyrophosphate by thiamine pyrophosphokinase in the cytoplasm, probably against a concentration due to a requirement of thiamine pyrophosphate for coenzyme functions. This problem could be approached by studying thiamine transport in intact isolated enterocytes.

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